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Award Number: **W81XWH-10-1-0228**

TITLE: **Optimizing Treatment of Advanced Prostate Cancer and Exploiting Mechanisms Driving Castration Resistance**

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REPORT DATE: **April 2011**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
04/30/2011	Annual	01 Apr 2010 – 31 Mar 2011

4. TITLE AND SUBTITLE
Optimizing Treatment of Advanced Prostate Cancer and Exploiting Mechanisms Driving Castration Resistance

5a. CONTRACT NUMBER
W81XWH-10-1-0228

5b. GRANT NUMBER
PC094259

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Castration resistant prostate cancer (CRPC) ubiquitously displays increased expression of the AR and AR target genes, reflecting the critical importance of this pathway in prostate cancer progression. As novel agents which potently inhibit the AR pathway become clinically available, it will be critical to understand how severe shutdown of the AR pathway impacts tumor cell biology and how this will alter the natural history of CRPC. This proposal investigates the impact of maximal intra-tumoral androgen pathway suppression on prostate tumor regression and recurrence, and seeks to determine whether specific resistance mechanisms identified at tumor progression will reflect the efficacy of androgen axis suppression and/or predict sensitivity to subsequent therapy. Our studies assess whether tumors recurring after the most stringent AR pathway blockade pharmacologically available will continue to retain a dependence on the AR axis or will exhibit an AR 'null' phenotype driven by non-AR directed resistance mechanisms. To date we have completed enrollment into our xenograft study of maximal androgen suppression, which uses the well defined LuCaP35 prostate cancer xenograft and three tiers of progressive AR axis suppression, as well as enrollment into one of two studies of high dose testosterone replacement therapy. Analysis of tumor growth and regression, as well as molecular analyses are ongoing and will be reported in years 2 and 3 of the award as per the Statement of Work.

15. SUBJECT TERMS
Castration resistant, intra-tumoral androgen, high dose testosterone

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

Castration resistant prostate cancer (CRPC) ubiquitously displays increased expression of the AR and AR target genes, reflecting the critical importance of this pathway in prostate cancer progression. This proposal investigates the impact of maximal intra-tumoral androgen pathway suppression on prostate tumor regression and recurrence, and seeks to determine whether specific resistance mechanisms identified at tumor progression will reflect the efficacy of androgen axis suppression and/or predict sensitivity to subsequent therapy. Our studies will assess whether tumors recurring after the most stringent AR pathway blockade pharmacologically available will continue to retain a dependence on the AR axis or will exhibit an AR 'null' phenotype driven by non-AR directed resistance mechanisms. As novel agents which potently inhibit the AR pathway become clinically available, it will be critical to understand how severe shutdown of the AR pathway impacts tumor cell biology and how this will alter the natural history of CRPC.

BODY

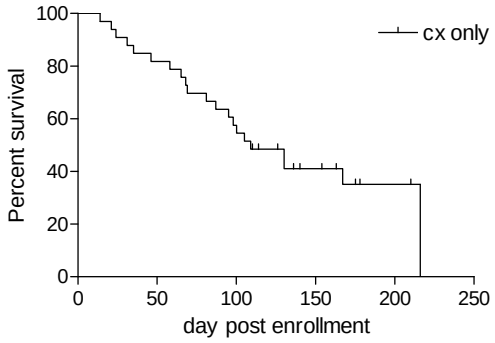
The primary research accomplishments associated with the Statement of Work for year one (months 1-12) are completion and/or initiation of the animal studies as listed in Technical Objectives 1 and 3 below. This report will detail the enrollment data for four of the five xenograft studies comprising this award (the fifth study will be performed during months 20-30 of the award). Evaluation of growth endpoints and molecular analyses have been initiated and will be further carried out and reported in years two and three of the award as per Technical Objectives 2 and 3 in the Statement of Work.

Technical Objective 1. Xenograft Studies of Maximal Androgen Suppression

1.1. castration (n=50 mice) months 1-12
1.2. castration+Casodex+Dutasteride+Ketocontazole (n=50 mice) months 1-18
1.3. castration+Vn-124 (n=50 mice) months 1-18

1.1 Enrollment into the castration only control arm has been completed, including n=5 tumors each harvested at days 7 and 21 after castration, and n=33 tumors harvested following castration recurrent re-growth (defined as re-growth to 750mm3). Preliminary analyses demonstrate an engraftment rate of 83% (predicted rate 80%), and that 65% of tumors recurred by day 200 following castration, thereby meeting the 60% incidence of re-growth in the control group that had been assumed for the power calculations (section 6a. in the ACURO animal appendix for these studies).

Figure 1. Time to castration recurrent progression (750mm3) in LuCaP35



1.2 and 1.3. The second arm of this study comprises maximal pharmacologic suppression of the androgen axis with currently available agents. This includes the CYP17A inhibition with ketoconazole, SRD5A1 and 2 inhibition with dutasteride, and the AR inhibition with the antiandrogen bicalutamide. Enrollment into this study has been completed, with n=5 and n=4 tumors harvested at the day 7 and day 21 time points, respectively, and n=32 tumors enrolled in

the long term treatment group. Tumor growth and regression data are still being collated and will be complete by month 18 as denoted in the SOW. The third arm of this study comprises treatment with the novel agent VN-124, which is a combined CYP17A inhibitor and androgen receptor antagonists. Enrollment into this study is presently ongoing and will be complete by month 18 as denoted in the SOW.

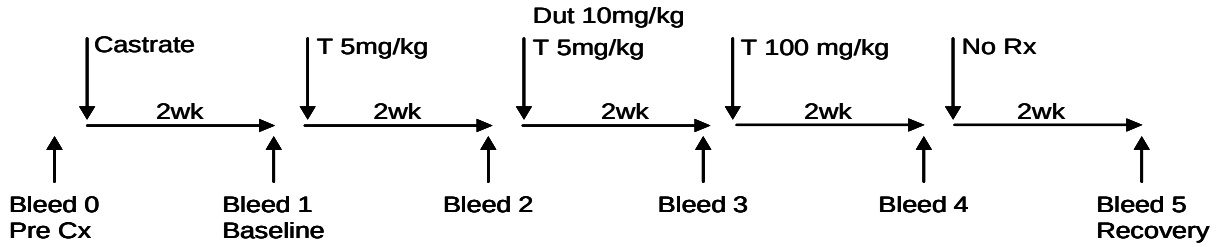
Technical Objective 3. Xenograft Studies of Testosterone Replacement

3a. Cohort 1 study with 4 arm randomization (n=100 mice), months 1-12
1. Placebo/vehicle control (n=20)
2. Testosterone low 5mg/kg/day (n=20)
3. Testosterone low + dutasteride (n=20)
4. Testosterone high 100mg/kg/day (n=20)

The goal of the studies in this Objective is to evaluate the differential sensitivity of tumors recurring after progressive AR inhibition to the repressive effects of supra-physiologic T replacement. Two studies are to be performed. The first study will evaluate the impact of testosterone treatment on the castration recurrent xenograft LuCaP 35V (Technical Objective 3a). The second study (Technical Objective 3b, in months 20-30) will be done with a LuCaP35V line that has been subjected to additional AR pathway inhibition and has then subsequently recurred as a 'super-resistant' line.

Pilot study to confirm appropriateness of testosterone dosing strategy. Prior to enrolling mice into the Cohort 1 study of testosterone replacement, a small pilot study using the proposed treatment regimens was performed to ensure that the replacement doses were yielding the desired serum testosterone levels. Due to baseline variability in circulating androgen levels, all mice were to be castrated and then treated with stepped doses of testosterone with or w/out dutasteride to yield either physiologic androgen levels, or the supra-physiologic androgen levels predicted to be necessary to cause testosterone-mediated growth inhibition. Five non-tumor bearing mice were serially treated with testosterone proprionate i.p. according to the schema in Figure 2.

Figure 2. Testosterone dosing schema to confirm achievement of appropriate serum levels



Measurements of serum androgen levels by mass spectroscopy are presented in Table 1 (below) and demonstrate achievement of physiologic serum T levels in the Low T + Dutasteride cohort (3.85 ng/ml +/- 084), and supra-physiologic levels in the High T cohort (75.1 ng/ml +/- 10.8).

3a. Enrollment into Cohort 1 has nearly been completed. To date n=20 mice have been enrolled into the no treatment control arm, and n=16 have been enrolled into each of the remaining 3 testosterone treatment arms. Completion of enrollment into this study is anticipated in the next month.

Table 1. Serum T levels after Low and High dose T replacement								
time point	serum T (ng/ml)	mouse number					average (stdev)	
		1	2	3	4	5		
0	Pre-Castration	9.19	2.47	7.23	5.34	7.21	6.29	2.53
1	Castration	0.01	0.01	0.01	0.02	0.01	0.01	0.01
2	Low T	0.95	0.75	1.09	0.73	0.79	0.86	0.15
3	Low T + Dut	3.71		4.37	4.62	2.73	3.85	0.84
4	High T	62.9		88.8	77.1	71.6	75.1	10.8
5	Recovery	0.11	0.07		0.14	0.05	0.09	0.04

KEY RESEARCH ACCOMPLISHMENTS

- Enrollment into 3 xenograft studies which will evaluate the impact of maximal androgen suppression on castration recurrent prostate cancer growth
- Confirmation of anticipated serum testosterone levels after low and high dose testosterone replacement therapy
- Enrollment into a 4 arm study which will assess the sensitivity of CRPC to physiologic and supra-physiologic testosterone replacement therapy
- Ongoing collection of tumor growth and regression data, and of tumor tissue for molecular studies

REPORTABLE OUTCOMES

A tissue repository comprising fixed and frozen tissues from the above xenograft studies is being generated. The fixed tissues will additionally be used to generate a tissue microarray resource.

CONCLUSION

As discussed, the primary research accomplishments associated with the Statement of Work for year one are completion and/or initiation of the animal studies. To date, analysis of these studies is ongoing and thus presentation of results or implications of the completed research is not yet available. We anticipate these studies will yield novel data on the ability of maximal pharmacologic androgen suppression to impact tumor androgen levels and castration recurrent growth, as well as the impact of supra-physiologic testosterone treatment on CRPC growth inhibition.